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The Effect of Vitamin D on Blood Pressure at Rest
and in Response to a Peak Graded Exercise Test

Braden Armstrong

B.S, University of Massachusetts Boston, 2010

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APPROVAL PAGE

Master of Science

The Effect of Vitamin D on Blood Pressure at Rest
and in Response to a Peak Graded Exercise Test

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Chapter 1 – Introduction

Background and Significance

Cardiovascular disease (CVD) is the leading cause of death in the United States (US) being responsible for more than 800,000 (32.8%) deaths annually [1, 2]. Hypertension [systolic blood pressure (SBP) >140 mmHg and/or >90 mmHg diastolic (DBP)] is strongly associated with all cause and CVD mortality [3]. Approximately, 32.6% of US adults ≥ 20 yr have hypertension and 17.2% of US adults ≥ 20 yr are unaware of their elevated blood pressure (BP) [2]. One in four people with prehypertension (120 - 139 mmHg SBP and/or 80 - 89 mmHg DBP) will develop hypertension within 4 yr [4]. Pre-hypertension in individuals >60 yr is associated with a 1.5 to 2-fold risk of experiencing a major CVD event [2]. Individuals with normal BP (<120 mmHg SBP and <80 mmHg DBP) at 50 yr have a 90% risk of developing hypertension within their lifetime [4]. The estimated medical cost for treatment of hypertension is \$46.4 billion annually [2]. The economic impact of hypertension on the US health care system has made it a critical public health concern and led to a focus on the importance of prevention and improved management.

Accumulating evidence indicates insufficient levels of vitamin D (VitD) are associated with high BP [5-7]. The currently used and accepted classification scheme for serum VitD is displayed in Table 1. Martins et al. (2007) examined data from the third National Health and Nutritional Examination Survey (NHANES III) and found the prevalence of hypertension to be 30% higher among US adults in the lowest quartile of serum VitD (≤ 21 ng/ml when compared to the highest quartile (≥ 37 ng/ml) adjusted for age, sex, race, and body mass index (BMI) [10].

Table 1. *Classification scheme of serum vitamin D* [8, 9]

Vitamin D / 25(OH)D Status	ng/ml	nmol/l
Sufficient	≥ 30	≥ 75
Insufficient	20 - 29	50 - 72
Deficient	≤ 19	47
Severe Deficiency	≤ 10	25

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ng/ml, nanograms per milliliter, nmol/l, nanomoles per liter

Furthermore, Foreman et al. (2007) investigated the association between serum VitD levels and risk of incident hypertension among 613 men and 1,198 women (BP >140/90 mmHg) aged 40 –79 yr (92% Caucasian, 8% unspecified). They concluded the relative risk of developing hypertension over a period of 4 to 8 yr among those deficient in plasma VitD (<15 ng/ml) was 6.13 in men and 2.67 in women [11]. This evidence suggests there is an association between lower levels of VitD and increased risk of hypertension; however, not all investigators reach these same conclusions [7, 10, 12-18].

Jorde et al. (2010) examined the cross-sectional relationship between levels of serum VitD and resting BP over 12 yr among 2,385 healthy men and women ≥ 25 yr who did not use BP medication (mean BP, 142/82 mmHg). After adjusting for sex, age, BMI and physical activity, the difference in SBP between the lowest and highest quartiles of serum VitD was not statistically significant. Jorde et al. concluded VitD status does not influence resting BP or predict future hypertension or elevated BP [13]. In 2003, Muray et al. examined the influence of VitD on resting BP among 590 healthy men and women and found SBP and DBP were higher in men with elevated levels of VitD, whereas there was no association between VitD levels and resting BP among women [14]. These findings suggest VitD may have a gender dependent effect

on BP. The existent literature on the association between VitD and resting BP is mixed and indicates a need for additional research [15-21].

Problem Statement

The evidence supporting an inverse relationship between VitD levels and risk of hypertension is controversial [19, 20]. Several studies have shown lower levels of serum VitD are associated with elevated resting BP [22-25]. However, other studies have shown no relationship between serum VitD and resting BP [13, 26-29]. Additionally, several studies have found higher levels of VitD are positively correlated with elevated resting BP [14, 30-32]. Further research is needed to resolve this controversy. The majority of cross-sectional studies examine individuals with insufficient and deficient levels of VitD. Thus, the purpose of this study will be to investigate the relationship between BP and individuals with VitD levels ≥ 34 ng/ml and those with VitD levels < 34 ng/ml. The median VitD level among the sample studied was 34ng/ml and therefore it was chosen as the cutoff value between groups.

To date, there are no published studies examining the relationship between VitD and the peak BP response to a graded exercise stress test (GEST). An exaggerated SBP response to peak exercise (≥ 210 mmHg in men and ≥ 190 mmHg in women) [33] is an independent risk factor for CVD and may predict future hypertension [33-36]. Miyai et al. [35], investigated the peak BP response to a GEST as a predictor of future hypertension among 726 men (42.9 ± 8.5 yr) who completed a GEST. The participants did not use BP medication and had normal resting BP (SBP < 120 mmHg and DBP < 80 mmHg). The average follow up was 4.7 yr after the initial visit. The cumulative incidence of hypertension increased progressively among individuals with higher percentiles of a peak SBP response to the GEST (relative risk 3.8). An exaggerated peak SBP response to a GEST was associated with a 3-to 4-fold greater risk of developing hypertension.

These results indicate that an exaggerated peak SBP response to a GEST is predictive of future hypertension [35, 37-39]. Further understanding of the relationship between VitD and the peak BP response to a GEST may be of clinical significance for the prevention, treatment, and control of hypertension [34, 35, 39].

This proposed study will examine the relationship between VitD and resting BP and will attempt to provide further insight into reasons for the contrasting results in the literature. It will also examine the effect of VitD on the peak BP response to a GEST. A major strength of this study is that it examines VitD status in a large cohort of healthy men and women spanning an age range of 61 yr. (20 – 81 yr.) [40].

Specific Aims

The aims of this study are to examine the relationship among serum VitD and resting BP and the peak SBP response to a GEST.

Specific Aim 1: To examine the influence of VitD on resting BP.

Hypothesis 1: Individuals with the lowest levels of VitD will manifest the highest resting BP.

Alternative Hypothesis: There will be no association between VitD levels and resting BP.

Specific Aim 2: To investigate the relationship between VitD and the peak SBP response to a GEST.

Hypothesis 2: Individuals with the lowest levels of VitD will manifest the highest peak SBP response to a GEST.

Alternative Hypothesis: There will be no association between VitD levels and the peak SBP response to a GEST.

Chapter 2 - Review of Literature

Introduction

Despite marketing claims from supplement manufactures such as Ortho Molecular Products that VitD can reduce BP and promote cardiovascular health, much is still unknown about the association between VitD and BP. A biologically plausible link between VitD and BP may be better understood through a greater comprehension of the mechanistic pathways in which VitD may affect the cardiovascular system. VitD is a unique nutrient that binds to receptor cells throughout the body and initiates hormonal responses that produce a myriad of feedback loops related to its health effects [41]. For example, the primary role of VitD and its active metabolites is to increase or maintain intestinal absorption of calcium and phosphate. If there is a disruption in the mineral levels within the blood stream due to either an excess of circulating VitD an increase in mineralization among vessel walls may occur [42]. Increased mineral deposits such as calcium on the vessel surface results in hardening and inflammation of the arterial walls [42]. Degradation of the arterial walls through the breakdown of elastin resulting from the buildup of calcium and phosphates compromises the structural integrity of the vessel. This mechanism reduces reactivity and elasticity within the vessel and ultimately increases BP [42]. VitD is believed to have a biphasic relationship with vascular calcification in both excess ($>30\text{ng/ml}$) and deficiency ($<15\text{ng/ml}$) [43]. In addition to vascular calcification many other mechanisms exist that influence the relationship between VitD and BP. The following review aims to investigate that relationship by providing a systematic analysis of the current body of literature and by examining the mechanistic links that have an influential role on both VitD and BP.

Literature Requirements

The current body of literature investigating the relationship between VitD and BP is largely characterized by cross-sectional studies that have resulted in inconclusive findings. For the purpose of this review, studies examining the relationship between VitD and BP that do not report mean VitD levels for subjects have been excluded [22, 24, 44-46]. Furthermore, studies consisting of children or adolescents, individuals with kidney disease, metabolic syndrome, diabetes, pregnancy or currently hospitalized for unspecified reasons have also been excluded from this review [28, 30, 47-49]. Studies examining the relationship between VitD and BP through the use of an intervention such as oral or UVB supplementation have been explored. However, in an effort to focus primarily on studies similar to the cross-sectional design of STOMP, which did not consist of a VitD intervention, only a brief overview of the findings from these intervention studies have been examined for informative purposes only and their findings have been excluded from this review [23, 32, 50-55]. Based on the criteria stated above, there are currently 19 cross sectional studies examining the effects of VitD on resting BP in adults >18 yr (Appendix A). VitD was found to lower resting BP in nine of the cross-sectional studies [19, 25, 56-62], while seven studies found no association between VitD and BP [13, 26, 27, 29, 63-65], and three studies found a positive relationship in which increased levels of VitD were associated with an increased resting BP [14, 31, 66].

To date, two meta-analyses [67, 68] and two systematic reviews [9, 69] meeting the inclusion criteria for this review have been conducted examining the association between VitD and resting BP. Study quality for the systematic reviews and meta-analyses contained in this literature review have been critiqued by the author of this review using the Preferred Reporting Items for Systematic reviews & Meta-Analyses (PRISMA) checklist [70]. The PRISMA

checklist consists of 27-items and a four-phase flow-diagram to ensure the complete reporting of systematic reviews and meta-analyses [70]. The 27 checklist items pertain to the content of a review or analysis, which include the title, abstract, methods, results, discussion and funding [70]. The flow diagram depicts the flow of information through the different phases of a review and lists the number of studies identified, included and excluded, and the reasons for exclusion [70].

In 2010, Burgaz et al. conducted a meta-analysis examining the blood concentration of VitD and its relationship with hypertension. Studies were included if they had a cohort, case-control or cross-sectional design with a hypertension outcome. The analysis consisted of 18 studies that meet the inclusion criteria. Participants included in the analysis were men and women between 18-96 yr. The concentration of serum or plasma 25(OH)D was the independent variable for the included studies and the outcome variable was hypertension [67]. Data extraction for the analysis was conducted independently by two investigators with the following relevant data being extracted from each study: sex, age, study design, method of measuring blood 25(OH)D concentration, mean 25(OH)D level, BP risk classification, and risk estimates (RR, HR, OR). The quality of the studies included in the meta-analysis was in accordance with the nine-star Newcastle-Ottawa Scale [71], which found no evidence of publication bias on any of the encompassing studies [67].

Of the 18 studies included in the analysis, Burgaz (2010) found the blood concentration of VitD was inversely associated with hypertension in 14 studies (odds ratio, 0.16-0.87). However, out of the 14 studies that found higher levels of VitD resulted in reduced BP, only 10 of those studies showed a significant association between VitD and hypertension ($P < 0.05$). The remaining four studies in the analysis showed a non-significant positive association between

25(OH)D and hypertension, indicating higher VitD levels are associated with higher BP (odds ratio, 1.01-1.28). Heterogeneity was statistically significant among all studies ($P=0.007$, $I^2=32\%$). The pooled odds ratio of hypertension for the highest category of blood 25(OH)D concentration versus the lowest category was 0.73 (95% CI: 0.63 – 0.84). The findings from this meta-analysis indicate an inverse relationship between blood 25(OH)D concentration and hypertension [67].

The meta-analysis conducted by Burgaz (2010) has many limitations, which indicate the need for further analyses. Few of the studies included in this meta-analysis were designed to specifically examine the effect of blood 25(OH)D concentrations on the risk of hypertension in a general population [11, 13, 25, 72]. Rather the primary aim for the majority of the studies included in this meta-analysis was determining the association between VitD and the metabolic syndrome. Therefore, it is conceivable that inadequate control of residual or unknown confounders may have implications in the observed findings. Furthermore, four studies [11, 60, 73, 74] included in the analysis used self-reported hypertension as outcome, which can lead to a false estimate of average BP and may bias the results toward underestimation or exaggeration of the hypertension risk estimates.

The findings of Burgaz, (2010) that suggested an inverse relationship between VitD and hypertension were refuted by two additional meta-analyses conducted by Witham et al. (2009) and Elamin et al. (2011). Witham et al. [20] examined eight randomized controlled trials ($n = 545$) on the effect of VitD supplementation on BP among individuals with hypertension. The authors found weak evidence to support a small non-significant effect of VitD supplementation on BP among individuals with hypertension [20]. Similarly, Elamin et al. (2011) analyzed 14 RCT's examining the relationship between VitD and BP. The 14 studies consisted of an

intervention cohort of 751 subjects and a control of 767 subjects. Statistical significance was not achieved in either SBP ($P=0.95$) or DBP ($P=0.33$). The reviewers concluded that the data available were unable to demonstrate an association between VitD and BP [75]. These meta-analyses examined the effect of VitD supplementation on BP among a variety of cohorts including individuals with chronic kidney disease, Type 2 diabetes mellitus, the metabolic syndrome, and osteoporosis and have therefore been excluded from this review [20, 75].

One of the many challenges when reviewing literature on the controversial topic of the association between VitD and BP is to discern the questionable studies with a less than desirable quality rating that repeatedly influence the results of meta-analyses and systematic reviews. For example, in 2010, Pittas et al. conducted a systematic review examining the association between VitD and cardiometabolic outcomes (Type 2 diabetes mellitus, hypertension, and CVD.) Although the systematic review consisted primarily of intervention studies, three longitudinal observational cohort studies with questionable methodology were also included in the systematic review. The three longitudinal studies conducted by Forman et al. (2007, 2008) and Wang et al. (2008) examined the association between VitD and hypertension using self-reported BP measurements to ascertain hypertension status [11, 73, 76]. Wang et al. (2008) examined the association between VitD and hypertension using both self-reported BP measurements and dietary intake as well as self-reported UVB exposure for VitD status. After an 8 yr follow up, Wang et al. (2008) found no association between VitD status and hypertension. However, Forman et al. (2007, 2008) who determined VitD status through measured 25(OH)D concentration found a significant association between lower 25(OH)D concentration and higher risk for incident hypertension after follow up at 7 and 8 yr (RR, 1.76 [CI, 1.27 to 1.24]) [11, 73]. Furthermore, Forman excluded women with BMI $>30 \text{ kg/m}^2$ and only included young women 32

to 52 yr which limits the generalizability of the results. The authors of the systematic review concluded that lower 25(OH)D concentration or VitD intake was associated with increased risk for incident hypertension, however not a single BP measurement was clinically obtained throughout the duration of all three studies. The findings from these three longitudinal studies demonstrate how questionable methodology can produce unconvincing results that impact the overall findings of the systematic review or meta-analysis.

Soon after the systematic review conducted by Pittas et al. (2010) suggested an inverse relationship between VitD status and increased risk for hypertension, Jorde et al. (2010) challenged these findings in a cross sectional study examining whether VitD status predicts future hypertension. In 2010, Jorde et al. [13] measured serum 25(OH)D status in 4,125 subjects from Tromso, Norway who did not use BP medication (aged 50-74 yr). This measurement was repeated 14 years later among 2,385 of the original subjects. When adjusted for sex, age, BMI, and physical activity, the researchers found serum 25(OH)D obtained in 1994 was not correlated with increased BP or the development of hypertension in 2008 (DBP, $r = -0.018$; SBP, $r = -0.012$) [13]. The findings of Jorde et al. (2010) that found no association between VitD and increased BP conflicts with much of the existing literature and indicates a need for additional research with exemplary methodology.

These findings were recently supported by Li et al. (2012) who conducted a cross-sectional study examining the association between VitD and BP among 1,428 Chinese adults (20-83 yr). Each participant's BP was measured three times by a physician using an automatic BP monitor after the individual had been seated for 5 minutes. The average of the three readings was used for analysis. Serum 25(OH)D was obtained from each participant and determined by radioimmunoassay. Mean levels of VitD among the cohort were 23 ng/ml, which were classified

as insufficient. 25(OH)D was not associated with BP in both adjusted and unadjusted models for both men and women ($P \geq 0.10$). Adjusted models were controlled for age, sex, BMI, alcohol intake, smoking, and family history of hypertension. The researchers concluded serum VitD is not independently associated with BP or risk of hypertension [63].

It is important to note that a primary limitation of cross sectional studies examining the association between VitD and BP is that lower VitD status may reflect nonspecific or undiagnosed chronic illnesses which may result in reverse causality [67]. These illnesses include, cancer, multiple sclerosis, Type 1 diabetes mellitus, depression, seasonal affective disorder, rheumatoid arthritis, psoriasis, tuberculosis and inflammatory bowel disease [8, 77]. Both longitudinal and cross-sectional studies have shown mixed results. Longitudinal studies such as the large population-based Norwegian Tromso study [13] did not find an association between VitD and BP after 14 yr. However, the Michigan Bone Health and Metabolism study conducted by Griffin et al. (2010) found significantly lower DBP among 413 women with VitD deficiency when compared to women with adequate VitD after 14 yr[27]. There were no differences in SBP between groups. Another longitudinal study conducted by Margolis et al. (2012) found no association between VitD and BP among 4,863 women over 7 yr. Similarly, cross-sectional studies have produced mixed results as well. A population-based study by Parikh et al. (2012) found that vitamin D was inversely correlated with both SBP ($p=0.02$) and DBP ($p<0.01$) among 701 individuals with a mean VitD level of 30 ng/mL, while Lihua et al. (2012) found no association between VitD and BP among 1420 individuals[63, 78].

Vitamin D and the Renin-Angiotensin-Aldosterone System

Numerous studies have suggested a correlation between VitD deficiency and activation of the renin-angiotensin-aldosterone system (RAAS) leading to increased BP. [79-84]. The RAAS is a regulatory cascade that plays a central role in maintaining BP and electrolyte homeostasis. The cascade of events triggered through stimulation of the RAAS that leads to the modulation of BP begins with the release of renin from the juxtaglomerular cells of the kidneys. The main purpose of renin is to cleave the amino acid angiotensinogen resulting in angiotensin I. Angiotensin I is further converted into angiotensin II (Ang II) by angiotensin-converting enzyme found primarily within the capillaries of the lung. Ang II is the key bioactive product of the RAAS acting as an endocrine, autocrine, and intracrine hormone and is responsible for vasoconstriction of the vessels resulting in an increase in BP [79-82]. Additional actions of Ang II include elevating sodium and water retention, which increases blood plasma volume and cardiac output, thus increasing BP [9]. Studies have shown that lower levels of biologically active forms of VitD such as 1,25(OH)₂D and 25(OH)D are associated with increased plasma renin activity and higher Ang II concentrations [83, 85, 86].

The metabolism of VitD and the mechanistic pathways in which VitD may lower BP are depicted in figure 1.

The primary mechanism linking VitD with an increase in BP is through suppression of renin within the juxtaglomerular cells [83, 84]. Li et al. [87] investigated the association between the VitD receptors (VDR) and renin and found that VDR knockout mice have a three-fold increase in renin expression and a 2.5 fold increase in plasma Ang II compared to a control group. Obstructing VitD synthesis in the VDR knockout mice led to an increase in renin expression that resulted into the development of hypertension and cardiac hypertrophy, whereas

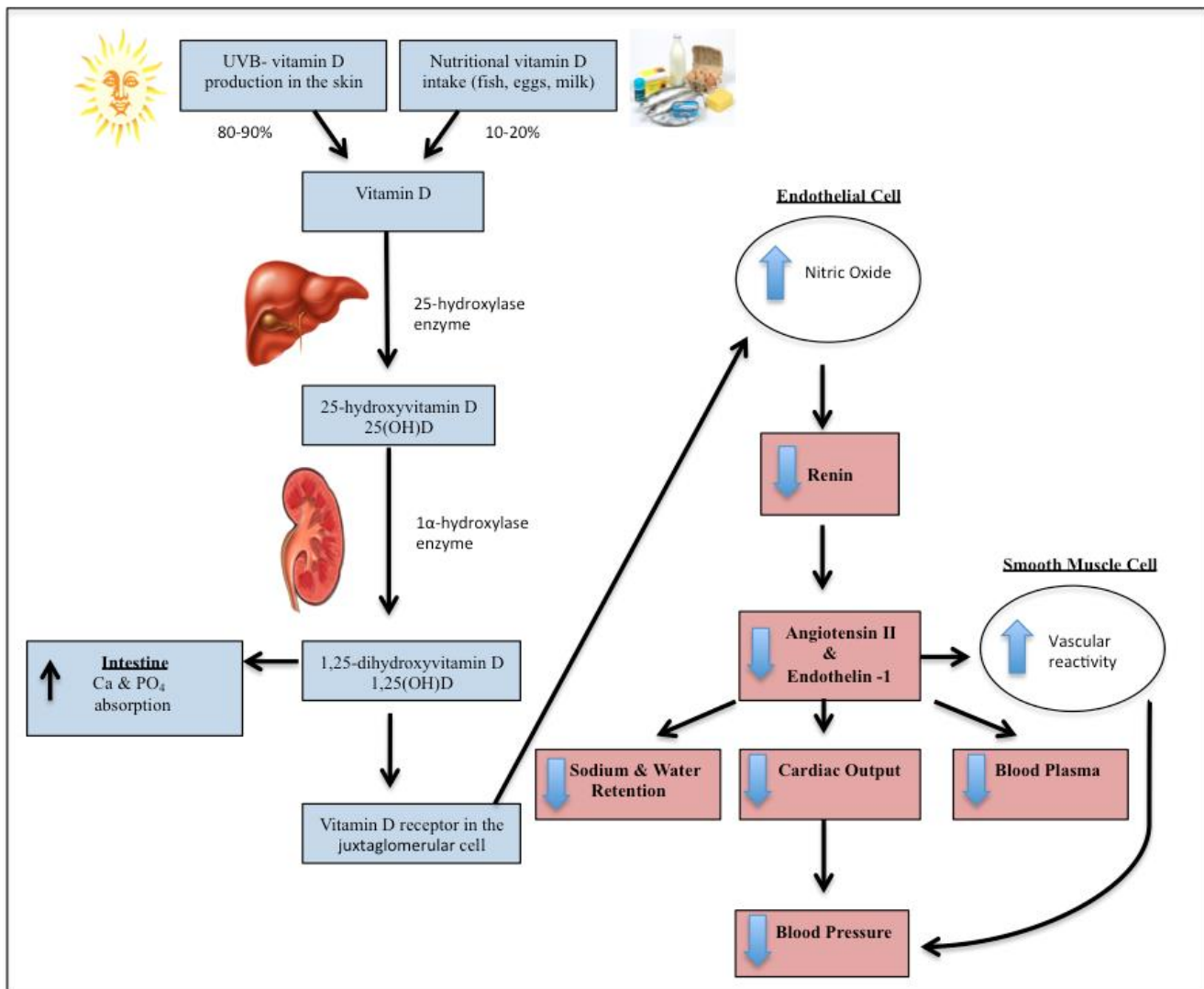
the control group received regular treatment of VitD and their renin levels and BP decreased [87]. Similarly, Zhou et al. [88] examined the cardiovascular effect of VitD among 1 α -hydroxylase knockout mice. 1 α -hydroxylase is an enzyme that catalyzes the hydroxylation of calcifediol to calcitriol, which is the bioactive form of VitD. The 1 α -hydroxylase knockout mice that were unable to synthesize calcifediol into an active form of VitD displayed enhanced RAAS activity in addition to hypertension and cardiac hypertrophy. All the aforementioned effects were diminished with the administration of the biologically active form of VitD, 1,25(OH) $_2$ D [88]. The collective work of both Li et al. and Zhou et al. has shown that the VDR acts to suppress renin gene expression and consequently modulate BP [87-89].

Among the several cross-sectional and prospective studies investigating the association of VitD deficiency and BP among humans, Forman et al [90] provided a mechanistic role of VitD in RAAS regulation. The researchers examined the relation between plasma VitD concentrations and both renin and Ang II levels among 184 individuals with normal BP. The individuals were placed on a high sodium diet for 3 to 7 days before the study because the range of renal plasma flow responsiveness in high sodium balance allows for more accurate detection of individual differences among participants. The researchers found that lower VitD levels (<15.0 ng/ml) correlated with higher levels of Ang II at baseline ($P = 0.03$). Plasma renin activity was also higher among individuals with insufficient levels of VitD, however not significantly ($p = 0.40$) [90]. These findings were confirmed in subsequent studies [83, 84] and indicate that VitD has an inverse association with both plasma renin and Ang II.

The RAAS has long been recognized as an important regulator of BP and electrolyte balance. Recent findings suggest that VitD is a potent endocrine suppressor of renin, which regulates the RAAS. VitD deficiency has been found to stimulate renin expression, whereas

sufficient VitD levels have inhibited renin synthesis. The role of renin is a key component when examining the association between VitD and BP. This association may provide an important mechanistic explanation for epidemiological data supporting the relationship between VitD deficiency and elevated BP.

Figure 1. *Vitamin D Metabolism and Mechanism of Action* [9]



Abbreviations: UVB, ultraviolet B; Ca, calcium; PO₄, phosphate

Vitamin D & Nitric Oxide

Another proposed hypothesis for the inverse association between VitD and BP is that serum VitD improves blood flow by enhancing vascular reactivity and endothelial function through the production and release of nitric oxide synthase (NOS) [47]. NOS is the key nitric oxide synthesizing enzyme responsible for converting nitric oxide into the biologically active form responsible for vasodilation and regulating vascular smooth muscle tone and reactivity [91, 92]. NOS opposes the vasoconstrictive properties of endothelin-1 and Ang II produced by the RAAS[91, 92]. The ability of VitD to enhance vascular reactivity and endothelial function, thus stimulating vasoactive substances such as NOS and suppressing the secretion of Ang II indicates the potential for VitD to have BP lowering effects [9].

Andrukhova et al. examined the association between VDR knockout mice and cell signaling within the endothelium to further understand the mechanisms by which VitD regulates vascular tone and cardiac function. The genetically modified mice were characterized by having lower bioavailability of nitric oxide due to reduced expression of endothelial NOS. VDR knockout mice they were fed a diet enriched with calcium, phosphate and lactose to ensure calcium homeostasis. After 12 months without cell signaling the mice had elevated BP, arterial stiffness, increased resistance of aortic blood flow, and structural remodeling of the aorta [93]. The authors found that reduced NOS production resulted in altered collagen and elastin content in the aorta by long term increases in mechanical strain [93]. Additionally, the authors found that VitD was a direct transcriptional regulator of endothelial NOS and that loss of VDR signaling increases arterial stiffness independent of activation of the RAAS. These findings indicate the importance of VDR signaling for maintaining efficient vascular function and may have important clinical implications in the treatment of CVD.

Vitamin D & Endothelial Dysfunction

Recent data indicate that low levels of VitD are associated with certain biomarkers related to endothelial dysfunction [94-96]. Endothelial dysfunction is observed through methods such as pulse wave velocity and carotid ultrasonography to determine if an increase in arterial wall thickness and stiffness has occurred[94]. Endothelial dysfunction is characterized by an increase of adhesion molecule expression, which can result in systemic vascular inflammation, decreased myocyte contractility and increased insulin resistance [94-96]. The primary biomarker that is indicative of platelet function and inflammation is C-reactive protein [94, 97, 98]. C-reactive protein is associated with inflammation and myofibroblast migration inside the vessel, which play an important role in the development of atherosclerosis. C-reactive protein is a crucial independent predictor of myocardial infarction, stroke, and vascular death [98].

Sypniewska et al. (2013) examined the association between VitD status and biomarkers of endothelial dysfunction in adults with hypertension. They included 158 individuals aged 35-65 yr with untreated or undiagnosed hypertension. No evidence of CVD or kidney disease was apparent in any of the individuals at the start of the study. Fasting venous blood was collected from the participants to determine VitD level. Echocardiography was measured to determine left ventricular mass and carotid ultrasonography was performed to assess carotid intima-media thickness, which is a measurement of atherosclerotic development. Additionally, carotid-femoral pulse wave velocity was measured as a measure of arterial stiffness and endothelial dysfunction. They found that individuals with VitD levels <20ng/ml had higher SBP than those with VitD >20ng/ml ($p=0.03$). C-reactive protein, was also higher among individuals with VitD <20ng/ml when compared to those with VitD >20ng/ml ($p=0.001$). Carotid intima-media thickness among individuals with VitD <20ng/ml was 0.81 ± 0.25 mm compared to 0.66 ± 0.15 in those with VitD

>20ng/ml ($p=0.001$). Pulse wave velocity was higher among those with VitD <20ng/ml (11.0 ± 2.0 m/s) compared to those with VitD levels >20ng/ml (8.5 ± 1.6 m/s) ($p=0.001$). These findings indicate that individuals with VitD levels <20 ng/ml may be more likely to develop a higher amount of biomarkers that promote endothelial dysfunction resulting in compromised vascular reactivity. Compromised vascular reactivity is directly correlated to an increase in BP [17, 21, 94, 98].

Vitamin D & Insulin Sensitivity

The endothelium regulates vascular tone by secreting vasodilators such as nitric oxide and vasoconstrictors such as endothelin [99]. However, individuals who are insulin resistant have an increase in the cytokine tumor necrosis factor alpha (TNF- α), which down regulates endothelial nitric oxide synthase and in-turn reduces production of the vasodilating nitric oxide [99]. This reduction of nitric oxide is directly association with an increase in BP [100]. Low levels of VitD are associated with hypertension and CVD and approximately half of all individuals with hypertension are insulin resistant [11, 22, 67, 101, 102]. Therefore, the associations between metabolic biomarkers such as fasting plasma insulin (FPI) and steady-state plasma glucose (SSPG) and BP have become a primary focus.

Abbasi et al. (2014) examined 140 individuals with hypertension to determine if there was a relationship between VitD levels, insulin resistance, and CVD risk factors. The sample consisted of mostly obese (58%) and overweight (34%) men and women with a mean age 55 yr. Of the total sample, 79% were being treated with medication for hypertension. After an overnight fast, FPI and SSPG were collected via catheter. The researchers found significant inverse correlations between VitD levels and FPI ($p=0.03$) and SSPG ($p=0.02$). These correlations remained significant after adjusting for smoking status and the use of

antihypertensive medication. Additionally, the researchers found that individuals with insufficient levels of VitD ($>20.3 \text{ ng/ml} \pm 1.4$) had SSPG levels $>229 \text{ mg/dl}$ and FPI levels of $19.2 \text{ } \mu\text{U/ml}$ classifying them as insulin resistant [17]. Insulin resistance as determined by SSPG levels showed a significant positive correlation among this sample to several CVD risk factors such as BMI ($p<0.001$), hypertension ($p<0.001$), heart rate ($p=0.008$), FPI ($p<0.001$), triglycerides ($p<0.001$), high-density lipoprotein cholesterol ($p<0.001$), and low-density lipoprotein cholesterol ($p<0.001$). SBP and DBP were not significantly associated with SSPG levels ($p>0.05$). However, insulin resistance is an independent predictor of hypertension. The findings of this study suggest that individuals with lower levels of VitD are more likely to have elevated SSPG and FPI levels classifying them as insulin resistant, which is significantly correlated to several other CVD risk factors including hypertension [17]. Insulin resistance is known to disturb nitric oxide mediated vasodilation and endothelial functions through the increase of TNF- α , which can result in vascular damage and lead to increased risk for long-term CVD.

Vitamin D & Intervention Therapy

Much of the existing literature suggests that VitD deficiency may promote elevated BP and other cardiovascular complications [2, 15, 17, 21, 97, 103] suggesting the possibility that VitD supplementation could be a viable means of reducing BP. However, data from randomized control trials are limited and inconsistent [23, 32, 50-55]. In 2009, Witham et al. conducted a meta-analysis to determine whether VitD supplementation reduces BP. The analysis included eight randomized controlled trials (RCT) ($n = 545$) examining the effect of VitD supplementation on BP among individuals with hypertension (mean BP $148/87 \text{ mmHg}$, mean age 62.2 yr). All studies were independently reviewed by two researchers according to a

predetermined protocol and found significant heterogeneity between studies ($I^2=62\%$, $P=0.01$). The studies showed a non-significant reduction in SBP among individuals with hypertension in the VitD group when compared with placebo (-3.6 mmHg SBP, $P = 0.1$) and a small yet statistically significant reduction in DBP (-3.1 mmHg DBP, $P = 0.01$). These findings indicate that VitD therapy is not an effective or beneficial way of reducing SBP among hypertensive individuals. The findings of Witham et al. were further supported by a meta-analysis conducted by Elamin et al. (2011).

In 2011, Elamin et al. analyzed 51 RCT with the primary aim of examining the relationship between VitD and cardiovascular outcomes (death, stroke, myocardial infarction). Secondly, they examined the effect of VitD D on CVD risk factors (i.e., BP, glucose and lipids). Reviewers worked both independently and in pairs to extract study characteristics and outcomes of interest in all eligible trials of moderate quality ($\kappa=0.80$). Of the 51 RCT, 14 studies examined the effect of Vit D on BP. The 14 studies consisted of an intervention cohort of 751 subjects and a control of 767 subjects. Subjects were predominantly unhealthy, older men and women ≥ 60 yr at risk of mortality (RR, 0.96), stroke (RR, 1.04), and myocardial infarction (RR, 0.96). The reviewers concluded that the data available were unable to demonstrate an association between VitD and SBP ($P= 0.95$) or DBP ($P=0.33$), however heterogeneity was significant ($I^2=61\%$). [75].

More recently in 2014, Arora et al. conducted a double-blind RCT to determine if VitD supplementation could be a viable intervention to reduce BP. The multicenter study examined 383 individuals aged 18 to 50 yr (mean age, 36 ± 10 yr.) with VitD levels ≤ 25 ng/ml (median VitD, 15.3 ng/ml) and a SBP between 120-159 mmHg. Participants were randomized to high-dose (4,000 IU/day) versus low-dose (400 IU/day) oral VitD supplementation for 6 months.

Follow-up visits occurred every 2 months until the end of the study and each visit consisted of 4 BP measurements. Upon conclusion of the study the researchers found no significant difference in the mean 24-hour SBP between either the high dose group (-0.8 mmHg) or the low dose group (-1.6 mmHg) ($p=0.71$) [55]. The findings of Arora et al. (2014) are supported by two additional studies [104, 105].

Larsen et al. (2012) examined 112 individuals with hypertension (mean age 61 ± 10 yr) who were randomized to 3,000 IU/day of VitD supplementation or a placebo for 5 months. The researchers found a nonsignificant reduction in 24 hour ambulatory BP among the intervention group (-3 mmHg, $p=0.26$) compared to the placebo group (-1mmHg, $p=0.18$). The findings of Larsen et al. were supported by a follow up study by Witham et al. (2013) examining the change in BP, arterial stiffness, and endothelial function after 1 year of VitD supplementation among 159 elderly individuals (mean age 77 yr) [104]. The groups were given 100,000 IU of oral VitD or placebo every 3 months for 1 year. VitD levels increased 10 ng/ml among the intervention group at 6 months and plateaued for the remainder of the year compared to placebo ($p<0.001$). Although, VitD levels increased among the intervention group there was no significant effect on BP at 1 year (SBP, $p=0.58$; DBP, $p=0.06$). The results from intervention studies that aim to determine if VitD has clinical significance as a potential therapy among individuals combating elevated BP have been largely unsuccessful. Currently the tolerable upper intake level for VitD for ages 9-71 yr is 4,000 IU/day, however a review by Holick et al. found VitD toxicity to occur over several months with a daily dosage of 50,000 IU [8, 106]. With a range of 45,000 IU/day between current tolerable upper intake and documented VitD toxicity future intervention studies need to be conducted with an emphasis on dosage because it is entirely plausible that 4,000 IU/day is not a dosage that will warrant a cardiovascular effect.

Vitamin D and Peak Blood Pressure

BP changes in response to a graded exercise stress test (GEST) have been found to correspond to BP changes initiated by daily physical stress conditions and are therefore a valuable predictor of future hypertension [107, 108]. Considerable increases in BP during exercise have been found to increase risk of left ventricular hypertrophy, myocardial infarction, cerebrovascular stroke, and cardiovascular death [107-111].

In 2007, Gupta et al. investigated whether the SBP response to a GEST is predictive of cardiovascular mortality among 6,145 individuals. Participants were grouped according to their exercise-induced increases in SBP (Group A, ≤ 43 mmHg, $n = 3,062$) (Group B, ≥ 44 mmHg, $n = 3,083$). BP was measured manually before, during and after the GEST. All participants underwent the GEST with a mean follow up of 6.6 ± 3.7 yr. Multivariate analysis was used to adjust for baseline differences between the two groups. During the follow-up, 676 individuals died of cardiovascular causes with an annual cardiovascular mortality of 1.6%. Cardiovascular mortality was significantly higher in group A (13.7%) than in Group B (8.2%) ($p < 0.001$). After adjusting for baseline differences in the two groups using multivariate analysis, an increase in SBP of < 44 mmHg was a significant predictor of mortality ($p < 0.05$). An increase in SBP ≥ 44 mmHg from baseline was associated with better survival over long-term follow up even in patients with a history of hypertension. This study demonstrates that an increase in SBP with GEST predicts cardiovascular mortality independent of age and exercise capacity. These results were further supported by the work of Farah et al. in 2008.

Farah et al. (2008) examined whether an exaggerated BP response to a GEST was predictive of future hypertension among 30 individuals with normal baseline BP ($< 140/90$ mmHg). The subjects performed a maximal GEST on a treadmill following the Bruce protocol

and were then divided into two groups. Group one consisted of 13 individuals who displayed exaggerated BP during the GEST (SBP \geq 210 mmHg, DBP \geq 105 mmHg) and group two consisted of 17 individuals who maintained a normal BP response to maximal exercise (SBP \leq 210 mmHg, DBP \leq 105 mmHg). More individuals in group one developed hypertension than those in group two ($P < 0.01$) at the 2 yr follow up (10 vs 1, $p < 0.001$). Of the participants that displayed an exaggerated BP during the GEST, 84% of them had developed hypertension by the 2 yr follow up [112].

The results of Farah et al. (2008) and Gupta et al. (2007) are consistent with those of Miyai et al. [35], who investigated the peak BP response to a GEST as a predictor of future hypertension among 726 men (42.9 ± 8.5 yr). The participants who were not taking any BP medication and had normal resting BP (SBP < 120 mmHg and DBP < 80 mmHg) were followed for a hypertensive outcome for 4.7 yr. The cumulative incidence of hypertension increased progressively among individuals with higher percentiles of a peak SBP response to the GEST (relative risk 3.8). An exaggerated peak SBP response to a GEST was associated with a 3-to 4-fold greater risk of developing hypertension. Progression of hypertension was found in 114 individuals (15.4%). These results indicated that an exaggerated peak SBP response to a GEST is predictive of future hypertension [35, 37-39].

However, these findings were challenged when Manolio et al. [33] examined the 5 year risk of developing hypertension among individuals who displayed an exaggerated BP response to peak exercise. In a population-based study 687 men and women (18-30 yr) with normal resting BP displayed an exaggerated BP response to a maximal GEST (SBP > 210 mmHg in men, SBP $190 >$ mmHg in women). The individuals who displayed an exaggerated BP response to peak exercise had increased their resting SBP an average of 5 mmHg and DBP 1 mmHg ($p < 0.005$) at

the 5 year follow up. Furthermore, they were 1.7 times more likely to develop hypertension when compared to those who displayed normal BP responses ($p < 0.001$). However, after multivariate regression analysis was conducted there was no longer a significant association between exaggerated BP response to a GEST and a 5 yr risk of developing hypertension [33].

The studies by Farah et al., Gupta et al. and Miyai et al. found that relative risk of hypertension and cardiovascular mortality among individuals with normal BP was greatly increased if they exhibited an exaggerated BP response to exercise. These findings indicate the importance of early detection through a GEST to reduce the risk of developing future hypertension. Among the current literature examining the association between VitD and BP none have yet to examine the relationship between VitD and the peak BP response to a graded exercise stress test (GEST).

Conclusion

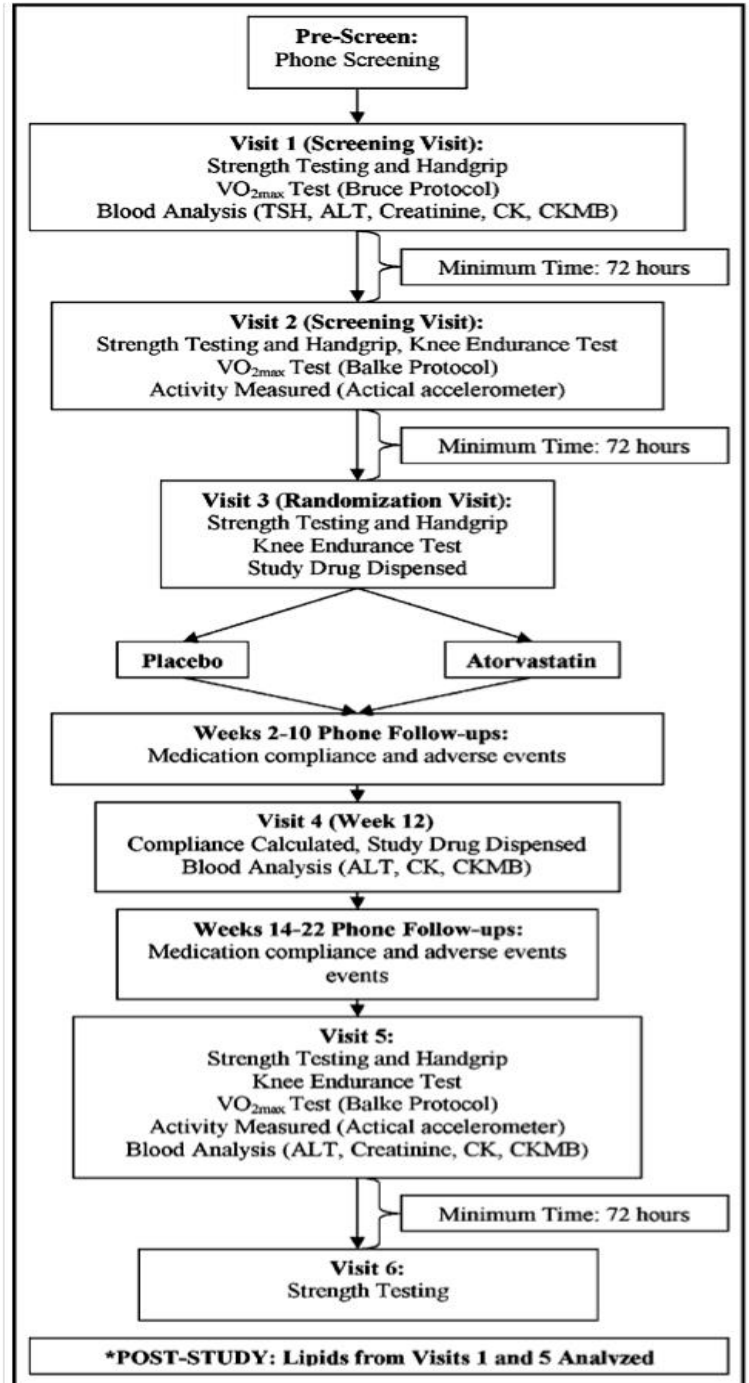
VitD is a unique nutrient that binds to receptor cells throughout the body and initiates hormonal responses that produce a myriad of feedback loops related to its health effects. VitD can be viewed as the catalyst in many chain reactions that initiate several effects on the cardiovascular system. Much of the cross-sectional data suggest a strong association between VitD and BP, which is supported by several biologic pathways such as the release of nitric oxide from endothelial cells and the suppression of renin. However, longitudinal and RCTs have produced mixed or weak findings suggesting a relationship between VitD and BP. Several of these studies evaluate BP as a secondary outcome and use self-reported BP measurements. Additionally, most intervention studies used a low-dosage of VitD or included individuals with well-controlled hypertension. Findings that suggest VitD is effective for lowering or normalizing BP have been largely inconsistent and contradictory. Therefore, it is premature to conclude that

maintaining optimal VitD levels will provide beneficial results when trying to reduce or normalize BP.

Chapter 3 - Methods

This proposed sub-study investigated the effects of VitD on BP at rest and in response to a GEST using data from a larger study entitled, “The Effects of Statins on Muscle Performance” (STOMP). This sub-study used the methods and data only from STOMP prior to randomization. STOMP was funded by the National Institute of Health (NIH) (1R01HL081893-01A2) and conducted by researchers at Hartford Hospital, the University of Massachusetts, Amherst, and the University of Connecticut, Storrs. The institutional review boards at the three study sites approved the experimental design of STOMP which has been described in detail elsewhere (Figure 1) [40, 113]. STOMP was a double blind, placebo-controlled randomized trial designed to examine the association between atorvastatin (Lipitor) and the incidence of statin-induced muscle myopathy among 440 healthy men and women >20 yr over a 6 month period. Secondly, STOMP investigators examined the effects of statin on

Figure 1. Overview of STOMP study.



TSH, thyroid stimulating hormone; ALT, alanine aminotransferase; CK, creatine kinase; CKMB, creatine kinase myocardial band

skeletal muscle strength,
endurance and aerobic exercise performance.

Subjects

Subjects for the larger STOMP study were recruited through study flyers, email announcements, and radio/newspaper advertisements. STOMP volunteers included 216 women and 203 men with a mean age of 44.1 ± 16.1 yr. Subjects signed an informed consent approved by the Institution Review Boards at each of the testing sites.

Exclusionary criteria were individuals who had: (a) diabetes mellitus; (b) hyper- or hypothyroidism; (c) any kind of surgery or injury to the knees or hips that would prevent the individual from exercising vigorously on a treadmill; (d) history of or current treatment with cholesterol-lowering medications; (e) a heart condition that required medication or a restriction of activity (e.g., cardiac disease and peripheral vascular disease); or (f) anyone with hepatic disease (alanine aminotransferase $> 2\times$ the normal limit) or renal disease (creatinine $> 2\text{mg/L}$) as determined by the blood sample obtained during visit 1 (V1). Additionally, individuals that experienced occult cardiac ischemia during the physician-supervised treadmill test in V1 were excluded from the study [40, 113]. Individuals using antihypertensive medications were included as long as their BP was controlled ($<140/90$ mmHg), and they had been on the antihypertensive medication for at least 3 months.

Study Overview

STOMP consisted of six visits to the laboratory over a period of approximately 6 months, however; only the visits that pertain to this study will be discussed in the overview. During visit 1 subjects provided an initial blood sample after fasting for 12 hours. The season in which the

blood samples were collected was recorded to account for potential seasonal variation of VitD levels. Total body VitD status was determined by measuring serum 25(OH)D, which is a measurement of the combined total of vitamin D2 and D3 levels in blood serum. Subjects then underwent a brief physical examination that included anthropometric and resting BP measurements as well as a physician supervised maximal GEST using a Bruce protocol with electrocardiographic monitoring. Study visit 2 occurred a minimum of 72 hr after visit 1 and included a maximal GEST using the modified Balke protocol to measure cardiorespiratory fitness. Approximately 90 days after visit 2 subjects provided another blood sample in a non-fasted state. After another 90 days subjects provided a final blood sample after fasting for 12 hr and then conducted a maximal GEST using the modified Balke protocol to measure CRF [40, 113, 114].

Body Composition

During visit 1 the subject's height and weight were measured and recorded to calculate BMI (kg/m^2). Height was measured using a wall-mounted tape measure while the subject stood erect with his or her head in the neutral position. Weight was measured using a calibrated balance beam scale. Prior to measuring height and weight, subjects were instructed to remove their shoes and any heavy or bulky clothing, such as a jacket or sweatshirt.

Blood Analyses

During visit 1 blood was collected from an antecubital vein without stasis by venipuncture. The blood samples sat at room temperature for a minimum of 10 minutes before being centrifuged at 3400 rpm for 15 minutes (VanGuard V6500, Hamilton Bell Co., Inc., Montvale, NJ, USA). After the blood samples were centrifuged, 1 mL aliquots of serum were obtained and shipped to Clinical Laboratory Partners in Hartford, CT where serum 25(OH)D

levels were obtained. This measurement was determined using a standard enzyme-linked immunosorbent assay protocol (ELISA) (Clinical Laboratory Partners, Newington, Connecticut). The ELISA protocol was used to determine 25(OH)D status by affixing an unspecified amount of 25(OH)D to a surface and then applying an anti-25(OH)D antibody over the surface. After an overnight incubation period the 25(OH)D antibody coupled to the enzyme. The chemical tetramethylbenzidine, which contains the enzyme's substrate was then added. The subsequent reaction produces a color change in the substrate which was used to determine serum 25(OH)D status [115]. A Clinical Laboratory Partners quality management team actively monitored performance indicators during specimen collection, analysis and reporting. The intra- and inter-assay coefficients of variation for this analysis were 5.3% and 4.6% respectively.

Peak Cardiopulmonary Graded Exercise Stress Test

During visit 2, CRF was measured with a peak GEST on a treadmill using a modified Balke protocol [40, 113, 114]. Prior to the GEST the subjects sat quietly for 5 minutes to allow for breath-by-breath analysis of expired gases (CO_2 , O_2 and V_E) via an open circuit respiratory apparatus (Parvomedics TrueOne 2400 metabolic cart, Parvomedics Corp, Sandy, UT, USA). After the 5 minutes of seated rest the subjects began the protocol by walking on the treadmill for 2 minutes at a speed of 2 miles per hour and a 0% treadmill incline. After the initial 2 minutes, the speed of the treadmill was increased until the subject was running at a speed at which they could comfortably maintain a steady state for at least 30 minutes. Treadmill speed was kept constant throughout the remainder of the test, however, after every minute the incline of the treadmill was increased 1%. VO_2 peak was determined directly during peak exercise. Heart rate (HR), BP and the individual's rate of perceived exertion (RPE) [116] was recorded every third minute of the GEST [113]. HR was recorded using a Polar[®] HR monitor (Polar Electro[®],

Kempele, Finland). RPE was recorded on a 6 to 20 point Borg scale. Criteria for GEST termination were: (a) an overall RPE ≥ 17 ; (b) a plateau in oxygen uptake; (c) a respiratory exchange ratio >1.1 ; (d) achievement of the age predicted maximum HR; and/or (e) termination by the subject due to fatigue or discomfort [113]. Monitoring of vital signs, RPE and expired gas collection continued for 6 minutes while the subject began active recovery by walking on the treadmill at a pace of 2.5 mph for 3 minutes then 2.0 mph for 3 minutes.

Blood Pressure

Prior to the GEST, resting BP was obtained after the subject sat upright with their back and arms supported for 5 minutes. BP was measured manually in the right arm positioned at heart level by auscultation using a mercury sphygmomanometer (Trimline™, PyMaH Corp., Somerville, NJ, USA), a Trimline BP cuff (Omni Kuff®, Latex Free, Universally connection BALANCED® design, Trimline Medical Products, Somerville, NJ, USA) and a Cardiology stethoscope (3M™ Litman® Lightweight II SE, St. Paul, MN, USA). Exercising BP was recorded every 3 minutes and was measured at heart level with the subject's arm relaxed and not grasping the handrails. Peak SBP was confirmed as the greatest BP recorded 1 minute prior to the conclusion of the GEST or immediately post-exercise (within 30 second of ceasing exercise) if peak SBP could not be attained at 1 minute prior to the conclusion of the GEST. A trained member of the study personnel measured SBP in the right arm by auscultation. If multiple BP measurements were recorded within the 1 minute prior and immediately post-exercise then the last measurement taken was used as the peak SBP.

Data Administration

Data collected during STOMP was compiled in an online master database, which was maintained by the study coordinator, at Hartford Hospital, Hartford, CT. Investigators from

Hartford Hospital, the University of Connecticut and the University of Massachusetts, Amherst manually entered STOMP data into the online database. Access to the database was secured using confidential usernames and passwords and was limited to study personnel only.

Statistical Analyses

The statistical approaches used addressed each of the following specific aims of the study:

Specific aim 1: To examine the association of VitD on resting BP.

Specific aim 2: To investigate the relationship between VitD and the peak SBP response to a GEST.

Descriptive statistics (mean \pm SEM) were calculated on all study variables for the total sample and by VitD status. Pearson product-moment correlation coefficients tested the relationship among resting SBP, DBP, MAP, the peak SBP achieved on the GEST, and VitD prior to randomization. Multivariable regression analyses were used to test the influence of VitD on resting SBP, DBP, MAP and the peak SBP achieved on the GEST. Analysis of covariance (ANCOVA) was then used to test the strength of the relationship between BP and VitD status with gender, BP medication, and VitD supplementation as fixed factors and age, BMI, and VO₂ peak as covariates. The dependent variables for the analyses were: resting SBP, DBP, MAP and peak SBP. The independent variable was VitD status. VitD status was determined as being above or below the median VitD level for the sample for purposes of statistical analyses. A Chi-square was used to test the relationship between subjects who on VitD supplementation and those on BP medication.

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) Base 19.0 for Macintosh (SPSS Inc, Chicago, IL) and $p < 0.05$ was used as the threshold for significance.

Chapter 4 – Results

Descriptive Characteristics

Sample Characteristics

Descriptive characteristics are shown in Table 1. The sample for this sub-study ($n=417$) consisted of healthy, overweight, middle-aged men ($n=203$, 48.6%) and women ($n=214$, 51.3%) with optimal BP and poor physical fitness for individuals of their age [117]. Resting SBP ($p=0.01$), DBP ($p=0.05$), and MAP ($p=0.01$) were higher among men compared to women (Table 2). The peak SBP response to a GEST ($p<0.001$) was also higher among men than women (Table 3). Men had a greater BMI ($p<0.001$) and VO_2 peak than the women ($p<0.001$).

The sample as a whole had sufficient levels of VitD (Table 1), with women having higher levels than men (37.9 ± 15.1 ng/ml vs. 34.1 ± 11.2 ng/ml) ($p=0.004$) [8, 9]. However, 51.7% of the men and 44.9% of the women had VitD levels below the median of 34 ng/ml. Men in the VitD group below the median had a VitD level that was 17.7 ± 1.0 ng/ml lower than the men above the median ($p<0.001$). Women in the VitD group below the median had a VitD level 22.0 ± 1.4 ng/ml lower than the women above the median ($p<0.001$). Men in the VitD group below the median had a slightly lower VitD by 0.27 ± 0.8 ng/ml than the women in this VitD group ($p>0.05$). Men above the median had a VitD level 4.5 ± 1.5 ng/ml ($p=0.003$) lower than the women above the median. Appendix B contains scatterplots of VitD levels among the total sample and by gender.

Subjects in the VitD group below the median had a lower VO_2 peak [33.4 ± 9.7 vs. 34.3 ± 9.7 ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)] and slightly higher BMI (26.8 ± 4.9 vs. 26 ± 4.8 kg/m^2) and were younger in age (43.3 ± 14.7 yr. vs. 44.9 ± 17.3 yr.) compared to subjects with VitD above the median. Men in the VitD group below the median had a lower VO_2 peak by 1.4 ± 1.3 $\text{mL}/(\text{kg} \cdot \text{min})$, and

slightly higher BMI by $0.62 \pm 0.6 \text{ kg}\cdot\text{m}^{-2}$ compared to the men above the median, however, neither difference was significant. Women in the VitD group below the median had lower VO_2 peak by $1.6 \pm 1.2 \text{ mL}/(\text{kg}\cdot\text{min})$, and a slightly higher BMI by $0.77 \pm 0.6 \text{ kg}\cdot\text{m}^{-2}$ compared to the women above the median, however neither difference was significant. Men in the VitD group below the median had a higher VO_2 peak by $8.5 \pm 1.2 \text{ mL}/(\text{kg}\cdot\text{min})$ ($p<0.001$), and BMI by $1.8 \pm 0.6 \text{ kg}\cdot\text{m}^{-2}$ ($p=0.006$) compared to the women below the median VitD. Men in the VitD group above the median had a higher VO_2 peak by $1.4 \pm 1.3 \text{ mL}/(\text{kg}\cdot\text{min})$, and higher BMI by $0.62 \pm 0.6 \text{ kg}\cdot\text{m}^{-2}$ compared to the women above the median VitD, however neither difference was significant.

VitD supplementation was taken by 24.1% of men and 32.2% of women. Among individuals who took VitD supplementation 53% of men and 59% of women have VitD levels above the median. Pearson's Chi-squared revealed no association between VitD supplementation and VitD status among individuals in this sample. Of the total sample, 4.9% of the men and 4.2% of the women were taking antihypertensive medications. Among those taking antihypertensive medication, 77% of women and 50% of men were above the median. Of the individuals not taking antihypertensive medication 54% of women and 47% of men were above the median.

Seasonal influence on VitD levels was significant in univariate models, however when controlling for age, sex and BMI, seasonal was not significant ($p>0.05$). This finding is consistent with those of Grimaldi et al. (2012) who found vitamin D was positively associated with isometric and isokinetic arm and leg strength measurements in univariate models. However, when including additional covariates such as SBP, DBP, BMI, $\text{VO}_{2\text{max}}$ in multivariate analysis VitD did not have a direct impact on isometric leg strength [118].

Vitamin D and Resting Blood Pressure

Table 2 contains resting BP by the median VitD level for the sample as a whole and by sex. Among the total sample, SBP (4.7 ± 12.2 mmHg, $p < 0.001$), DBP (1.9 ± 8.7 mmHg, $p = 0.029$) and MAP (2.8 ± 8.4 mmHg, $p < 0.001$) were higher among those with VitD levels above the median compared to those below the median. Men with VitD below the median had a lower SBP by 5.9 ± 12.6 mmHg ($p < 0.001$), DBP by 3.2 ± 9.8 mmHg ($p = 0.04$), and MAP by 4.1 ± 9.9 mmHg ($p < 0.001$) than the men above the median VitD. Women with VitD below the median had a SBP 4.2 ± 13.8 mmHg ($p = 0.003$) lower than the women above the median. Within the VitD group below the median men had a higher SBP by 5.1 ± 1.7 mmHg ($p = 0.004$) and MAP by 2.6 ± 1.3 mmHg ($p = 0.04$) than the women below the median. Men above the median had a higher SBP by 6.7 mmHg, ($p = 0.003$), DBP by 3.8 mmHg, ($p = 0.007$) and MAP by 4.7 mmHg, ($p = 0.001$) when compared to the women above the median.

Vitamin D and the Peak SBP Response to a GEST

Table 3 contains the peak SBP response to a GEST by median VitD level for the sample as a whole and by sex. Among the total sample, subjects with VitD levels below the median had a peak SBP that was 6.3 ± 21.3 mmHg lower than those above the median ($p = 0.04$). Men below the VitD median had a peak SBP that was 8.4 ± 21.3 mmHg lower than those above the median ($p = 0.04$). Similarly, women with a VitD below the median tended to have a peak SBP that was 4.4 ± 19.4 mmHg lower than women above the median, however, this result did not achieve statistical significance ($p > 0.05$). Men above the median had a peak SBP that was 18.8 ± 2.8 mmHg higher than women above the median ($p < 0.001$). Men below the median had a peak SBP 14.8 ± 2.7 mmHg higher than women below the median ($p < 0.001$).

Correlates of Resting Blood Pressure and the Peak Systolic Blood Pressure Response to a GEST

The models that emerged from the multivariable regression of correlates of resting BP and the peak SBP response to a GEST are shown in Table 4. VitD (4.0%, $p=0.000$), age (5.2%, $p=0.000$), sex (4.0%, $p=0.000$), and BMI (6.4%, $p=0.000$) explained 17.8% of the variability in resting SBP. VitD (1.0%, $p=0.04$), age (2.7%, $p=0.001$), sex (1.0%, $p=0.045$), and BMI (5.9%, $p=0.000$) explained 10.9% of the variability in resting DBP. VitD (2.5%, $p=0.001$), age (4.4%, $p=0.000$), sex (2.4%, $p=0.002$), and BMI (7.4%, $p=0.000$) explained 16.0% of the variability in resting MAP. VitD (1.4%, $p=0.016$), age (2.5%, $p=0.001$), sex (10.0%, $p=0.000$), and BMI (3.0%, $p=0.001$) explained 15.8% of the variability in peak SBP response to a GEST.

Table 1. Subject Characteristics (Mean \pm SEM) among total sample and by sex and vitamin D level

<i>Variable</i>	<i>Total Sample (N=417)</i>	<i>Vitamin D < 34 ng/ml (N=203)</i>		<i>Vitamin D \geq 34 ng/ml (N=214)</i>	
		<i>Men (N=105)</i>	<i>Women (N=96)</i>	<i>Men (N=98)</i>	<i>Women (N=118)</i>
Age (yr)	44.1 \pm 0.8	42 \pm 1.5	44.6 \pm 1.4	45.2 \pm 1.7	44.7 \pm 1.7
BMI (kg·m ⁻²)	26.4 \pm 0.2 ††	27.7 \pm 0.5	25.9 \pm 0.5 †	27.1 \pm 0.4	25.1 \pm 0.5 †
Vitamin D	36.1 \pm 0.7 ††	25.5 \pm 0.5**	25.8 \pm 0.6**	43.3 \pm 0.8**	47.8 \pm 1.2 †**
VO ₂ peak (ml·kg ⁻¹ ·min ⁻¹)	33.9 \pm 0.5 ††	37.5 \pm 0.9	28.9 \pm 0.9 ††	39.0 \pm 0.9	30.6 \pm 0.8 ††
Blood Pressure Medication Use	19	5	2	5	7
Vitamin D Supplementation Use	118	23	28	26	41

BMI, Body Mass Index; VO₂peak , Peak Oxygen Consumption

† p<0.05, †† p<0.001 Men vs. Women

* p<0.05, **p<0.001 Vitamin D, <34 ng/mL vs. \geq 34 ng/mL

Table 2. Average adjusted resting blood pressure (Mean \pm SEM) among the total sample and by median vitamin D level and sex.

Resting Blood Pressure (mmHg)	Total Sample	Vitamin D <34 ng/mL	Vitamin D \geq 34 ng/mL
Total	<i>n=417</i>	<i>n=203</i>	<i>n=214</i>
SBP	118.9 \pm 0.6	116.6 \pm 0.9	121.3 \pm 0.9**
DBP	75.3 \pm 0.5	74.3 \pm 0.6	76.2 \pm 0.6*
MAP	89.9 \pm 0.5	88.4 \pm 0.6	91.2 \pm 0.6**
Men	<i>n=203</i>	<i>n=105</i>	<i>n=98</i>
SBP	117.2 \pm 0.8	119.0 \pm 1.2	124.9 \pm 1.2**
DBP	76.5 \pm 0.6	75.1 \pm 0.9	78.3 \pm 0.9*
MAP	91.6 \pm 0.7	89.7 \pm 0.9	93.8 \pm 0.9**
Women	<i>n=214</i>	<i>n=96</i>	<i>n=118</i>
SBP	113.0 \pm 0.8 ††	114.0 \pm 1.2 †	118.2 \pm 1.1 †**
DBP	74.3 \pm 0.6	73.6 \pm 0.9	74.5 \pm 0.9 †
MAP	88.6 \pm 0.6	87.1 \pm 0.9	89.1 \pm 0.9

Adjusted for age, body mass index, sex, V0₂, and BP medication use

BP, Blood Pressure; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MAP, mean arterial pressure; BMI, body mass index

† p<0.05, †† p<0.001 Men vs. Women

Table 3. Average adjusted peak systolic blood pressure (Mean \pm SEM) response to a GEST among the total sample and by the median vitamin D level and sex.

Peak Systolic Blood Pressure (mmHg)	Total Sample	Vitamin D <34 ng/mL	Vitamin D \geq 34 ng/mL
	<i>n</i> =277	<i>n</i> =139	<i>n</i> =138
Total	169.4 \pm 1.1	166.6 \pm 1.6	172.6 \pm 1.6*
	<i>n</i> =138	<i>n</i> =74	<i>n</i> =64
Men	177.1 \pm 1.6	173.6 \pm 2.3	182.0 \pm 2.5*
	<i>n</i> =139	<i>n</i> =65	<i>n</i> =74
Women	161.7 \pm 1.6 $\dagger\dagger$	158.8 \pm 2.0 $\dagger\dagger$	163.2 \pm 1.9 $\dagger\dagger$

Adjusted for age, body mass index, sex, V0₂, and BP medications

BP, Blood Pressure; SBP, Systolic Blood Pressure; GEST, Graded Exercise Stress Test

\dagger p<0.05, $\dagger\dagger$ p<0.001 Men vs. Women

* p<0.05, Vitamin D, <34 ng/mL vs. \geq 34 ng/mL

Table 4. Multivariable regression of correlates of resting BP and the peak SBP response to a GEST

<i>Dependent variable</i>	<i>Predictors</i>	β	<i>t</i>	<i>Partial r</i>	r^2	<i>P</i>
Resting systolic blood pressure	VitD	0.186	4.153	0.2	0.178	0.000
	Age	0.176	4.74	0.227		0.000
	Sex	-5.033	-4.015	-0.199		0.000
	BMI	0.677	4.153	0.254		0.000
Resting diastolic blood pressure	VitD	0.07	2.065	0.101	0.109	0.04
	Age	0.095	3.38	0.164		0.001
	Sex	-1.858	-2.008	-0.098		0.045
	BMI	0.487	5.068	0.242		0.000
Resting mean arterial blood pressure	VitD	0.109	3.217	0.157	0.16	0.001
	Age	0.122	4.356	0.21		0.000
	Sex	-2.916	-3.163	-0.154		0.002
	BMI	0.55	5.744	0.272		0.000
Peak systolic blood pressure	VitD	0.172	2.424	0.119	0.158	0.016
	Age	0.19	3.24	0.158		0.001
	Sex	-12.78	-6.599	-0.309		0.000
	BMI	0.668	3.317	0.161		0.001

VitD, vitamin D; BMI, body mass index

Chapter 5 - Discussion

Hypertension is a highly prevalent risk factor for CVD-related death [3]. More than a quarter of all adults ≥ 20 yr are living with hypertension and nearly a fifth of all adults ≥ 20 yr have elevated BP but remain unaware of it [2]. Even among adults (50 yr) with normal BP, there is a 90% risk of developing hypertension during their lifetimes [4]. Thus it is important to further our understanding of hypertension, what precipitates its incidence, and how it can be prevented.

One controllable variable that may influence BP is serum VitD levels. Accumulating evidence indicates the risk of hypertension is associated with VitD deficiency [5-7]. For example, the prevalence of hypertension was found to be 30% higher among people in the lowest quartile of serum VitD levels relative to those in the highest quartile.[10] Additional studies have supported these claims, reporting that lower serum VitD levels associate with elevated resting BP [22-25]. However, other authors have reported conflicting views. For example, several studies found VitD to have no effect on resting BP [13, 26-29] and still other studies found elevated VitD levels to correspond to increases in resting BP [14, 30-32].

We attempted to explore these discrepant findings in a sample of 417 healthy men and women who neither have VitD deficiency nor hypertension. Patients across the lifespan, age 20 – 81 yr, were divided by the median level of serum VitD (34 ng/ml) and the relationship between serum VitD and BP was evaluated. BP was measured both by resting evaluations and by peak SBP response to a GEST, as elevations in peak BP, particularly SBP, during a GEST can predict future onset of hypertension [33-39]. Owing to trends in the literature, we had anticipated finding individuals with the lowest serum VitD to manifest the highest resting BP as well as the highest peak SBP during a GEST. The major findings were that resting BP was significantly higher among individuals with VitD levels above the median compared to those below the median.

Among the subjects who achieved a peak SBP response to a GEST, a similar result was found: we recorded higher peak SBP values for subjects whose VitD levels were above the median ($p=0.04$).

Gender differences were also found. Overall, men displayed a higher resting SBP ($p=0.01$), DBP ($p=0.05$), and MAP ($p=0.01$) than women. Peak SBP during the GEST was also higher among men than women ($p<0.001$). Men with VitD levels below the median had lower resting SBP and DBP than those with VitD levels above the median. Similarly, women with VitD levels below the median had a lower resting SBP when compared to women above the median ($p=0.003$).

These findings are important because, while the previous literature was conflicted, the majority of the studies reported either no association between BP and VitD or the relationship to be inverse, whereby lower VitD levels correspond to higher BP [20, 67, 68, 75]. Three recent meta-analyses have analyzed the relationship between VitD levels and BP. In 2009, Witham and colleagues [20] investigated the effect of VitD supplementation on BP among men and women with hypertension, finding VitD to have a very weak, non-significant BP-lowering effect. Of note, all subjects in this meta-analysis had confirmed hypertension. In 2010, Burgaz and colleagues found VitD levels to be inversely associated with BP. However, the primary aim for the majority of the studies included in the Burgaz analysis was to determine the association between VitD and the metabolic syndrome and therefore included a cohort that would have been otherwise excluded from the present study due to health requirements. A second meta-analysis conducted by Elamin and colleagues. (2011) found no association between VitD status and BP among 1,518 individuals across 14 studies. However, this work included patients with chronic kidney disease, Type 2 diabetes mellitus, and the metabolic syndrome. Therefore, the findings

from both meta-analyses cannot be readily generalized to our subject population, which consisted of only healthy adults.

Moreover, many of the studies examining the association between VitD and BP have relatively low quality consequent of poor methodology. For example, it is not uncommon for studies to include self-reported BP measurements status [11,107,109] or estimate VitD status by scrutinizing dietary logs rather than collecting blood samples [109]. Studies with such methodological limitations are routinely included in the systematic reviews and meta-analyses examining the relationship between VitD and BP, and thus the conclusions of these reports may not be trustworthy. In addition to our subjects being healthy and our methods being sound, another explanation for the discrepant findings is the average VitD levels in our population. Most of the existing research has examined patients with VitD deficiency or insufficiency and their risk of hypertension. Thus the tested cohort typically has a mean VitD level <25 ng/mL. By comparison, the subjects in this study had a median VitD level of 34 ng/mL, so the question was not whether deficiency precipitates changes in BP, but if fluctuations within a normal range can also elicit change.

A possible physiological explanation for the elevated BP among people with increased VitD levels relates to its primary role: regulation of the intestinal absorption of calcium and phosphate. An excess of circulating VitD may disrupt mineral levels within the vasculature and lead to increased mineralization (e.g., calcium deposits) within the vessel walls. In turn, this can lead to vascular hardening and inflammation [42]. Furthermore, when calcium and phosphate levels build up, it can induce the breakdown of elastin, resulting in the deterioration of the arterial walls, reducing reactivity and elasticity, and ultimately increasing BP [42]. It is likely that VitD can stimulate vascular calcification not just during deficiency, but also during excess

(>30 ng/ml) [43]. One area that particularly warrants further investigation is in the gender roles. In our study, men displayed a higher resting SBP, DBP, and MAP than women as well as a higher peak SBP during the GEST. In the literature, the relative risk for developing hypertension among people deficient in VitD was higher in men [11] and there may be more to this relationship. Muray and colleagues (2003) found BP to be higher in men with elevated VitD levels, but no association between VitD and resting BP among women [14]. Further exploration into the effect of gender on VitD's relationship with BP may be revealing.

Limitations and Strengths

A primary delimitation posing a threat to the external validity is that STOMP was not designed to address the primary aims associated with this sub-study. The original objectives of the STOMP study did not include resting BP or the peak SBP response to a GEST as primary outcomes. Therefore, the peak SBP response to a GEST was obtained in 277 participants out of a total sample size of 417 individuals. A potential threat to internal validity exists among the consistency and reliability of data collection and reporting methods and procedures across the 3 data collection sites. Discordant interpretations during data collection and entry at the different sites are a potential weakness. To minimize this threat a standardized database, testing protocol and equipment were used at each site. Additionally, investigators responsible for data collection at each site participated in mandatory monthly meetings to discuss study progress and ensure investigators at each site were accurately following the study protocol, limiting the opportunity for bias during data collection and entry [40].

A third limitation is the possibility that the relationship between VitD and blood pressure was offset by the use of antihypertensive medications, as subjects were enrolled in the study as long as their BP was controlled (<140/90 mmHg), and they had not newly initiated treatment.

Antihypertensive medications may have offset the potential influence of VitD levels in some patients. There are several strengths to the STOMP study including the large sample of healthy men and women (n= 417) with a wide age range (20 – 81 yr.) The wide age range used in this STOMP sub-study allows for the findings to be generalized to a larger group of people.

An additional limitation is that dietary intake was not recorded or monitored for duration of the STOMP study. A dietary record of the quantity and percentage of macro and micronutrients consumed by the sample, such as calcium, may be an important covariate when examining the association between VitD and BP. VitD is significantly more effective in reducing SBP when supplemented with the proper amount of calcium. If an individual is calcium deficient then VitD alone is less likely to have a BP altering effect [41,46]. Additionally, sodium chloride (table salt) which has no association to VitD levels and a strong association to BP could have been an important covariate if recorded [86].

Lastly, the median VitD level among the sample was 34 ng/ml, which greatly exceeds the Vit D levels examined in the majority of previous literature. Therefore the findings put forward from this sub-study highlight a unique sample not well represented in previous studies examining the relationship between BP and VitD.

Conclusion

We found VitD levels to positively associate with BP among 417 healthy men and women across the lifespan who had a resting MAP of 90mmHg and median VitD of 34 ng/mL. BP was measured both at rest and during a maximal GEST, and the positive association with VitD levels was found in both scenarios. These results are atypical and would benefit from additional research. This is the first study to examine the association between VitD levels and the peak SBP response to a graded GEST and therefore offers preliminary data in that domain.

Appendix A.

Table 1.

Cross-sectional studies reporting an inverse association between vitamin D and blood pressure

Year	Author	N	Population	Mean Vit D (ng/ml)	Mean SBP	Mean Age	Mean BMI
2011	Deo et al.	2312	Healthy men and women	21.8	139	74	27.3
2011	Burgaz et al.	833	Healthy Caucasian men	27.7	140	71.8	25.8
2010	Kim et al.	324	Healthy men and women	18.3	138	65.8	23.7
2010	Zhao et al.	7228	Healthy men and women	23.2	120	47.4	28.7
2009	Gannage-Yared et al.	381	Healthy men and women	31	110	23.9	23.9
2009	Schmitz et al.	1334	Hispanic and African American men and women	14.8	NA	38.5	NA
2008	Rueda et al.	298	Obese men and women	19.1	NA	42.9	46.7
1997	Kristal-Boneh et al.	100	Male industrial employees	25.3	121	41.9	26.3
1994	Duprez et al.	25	Individuals with HTN	17.6	164	44.1	NA

Abbreviations: SBP, systolic blood pressure; yr, years; BMI, body mass index; ng/ml, nanograms per milliliter; HTN, hypertension; NA, not available

Table 2.

Cross-section studies reporting no association between vitamin D and blood pressure

Year	Author	N	Population	Mean Vit D (ng/ml)	Mean SBP	Mean Age (yr)	Mean BMI
2012	Li et al.	1420	Chinese men and women	23	124.3	47	23.2
2011	Scragg et al.	119	Healthy men	17	134	57	33
2011	Griffin et al.	559	Healthy females	23.5	119	37.7	26.8
2011	Chan et al.	939	Chinese males	31.2	142	72.8	23
2010	Jorde et al.	2385	Healthy men and women	21.5	139	56.2	26.2
1995	Scragg et al.	191	Non-medicated individuals with HTN	13.2	NA	70	NA
1995	Lind et al.	34	Non-medicated men	36.1	143	63	NA

Abbreviations: SBP, systolic blood pressure; yr, years; BMI, body mass index; ng/ml, nanograms per milliliter; HTN, hypertension; NA, not available

Table 3.

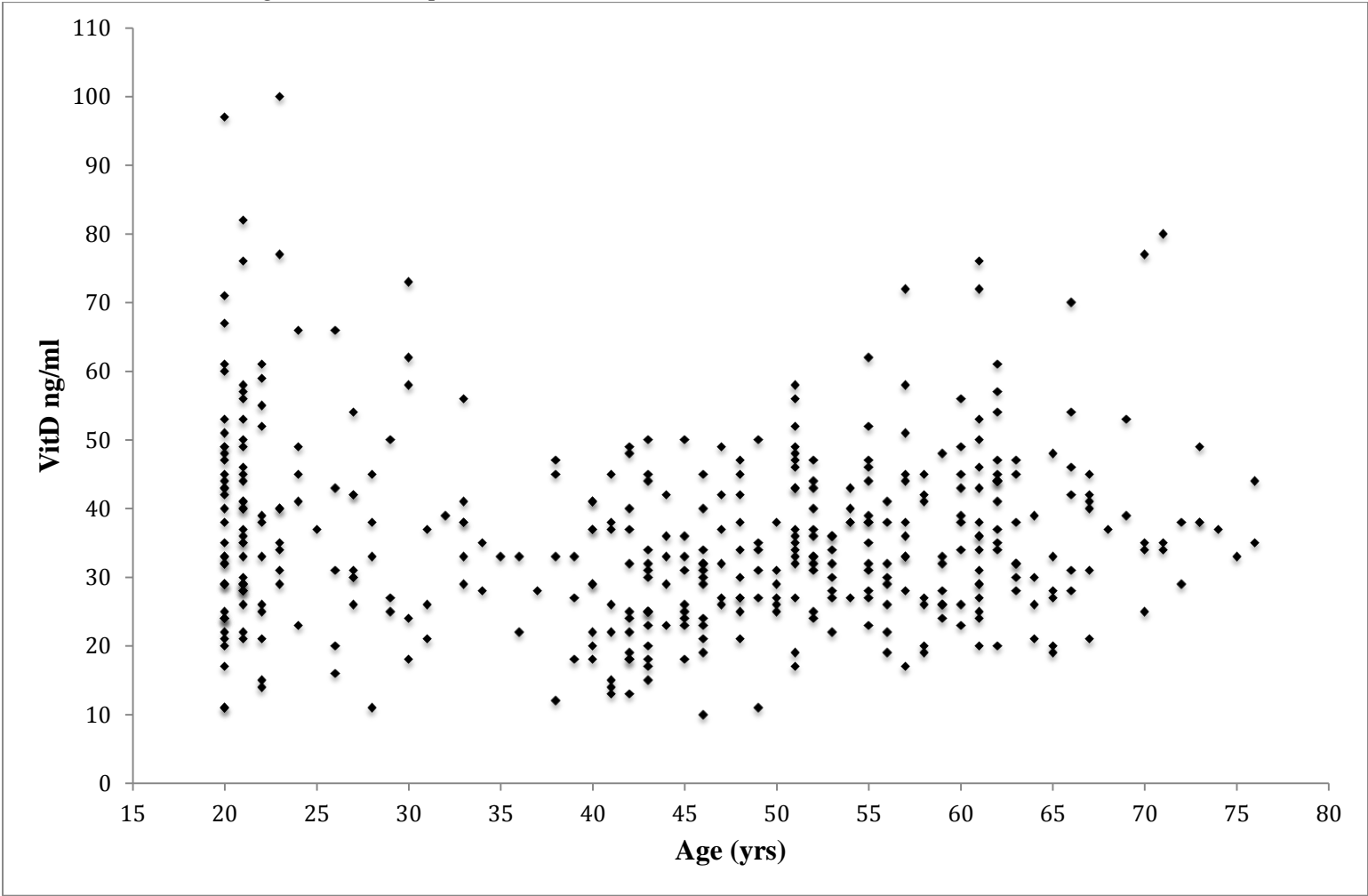
Cross-section studies reporting a positive association between vitamin D and blood pressure

Year	Author	N	Population	Mean Vit D (ng/ml)	Mean SBP	Mean Age (yr)	Mean BMI
2003	Murray et al.	590	Healthy men and women	22.8	122	36	24.7
1988	Sowers et al.	373	Healthy women	24.3	128	NA	NA

Abbreviations: SBP, systolic blood pressure; yr, years; BMI, body mass index; ng/ml, nanograms per milliliter; HTN, hypertension; NA, not available

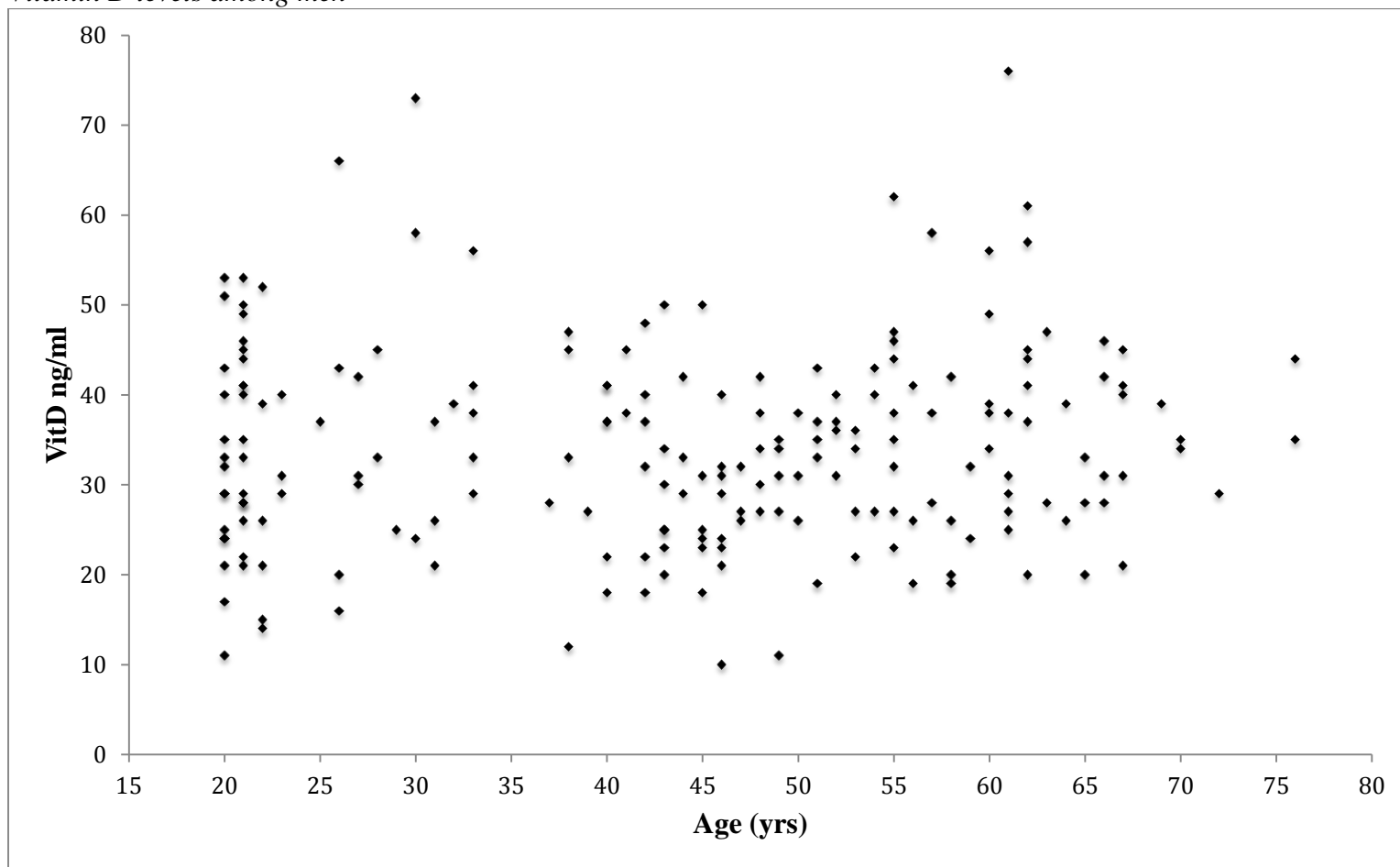
Appendix B.

Chart 1.
Vitamin D levels among the total sample



VitD, vitamin D; ng/ml, nanograms per millileter; yrs, years

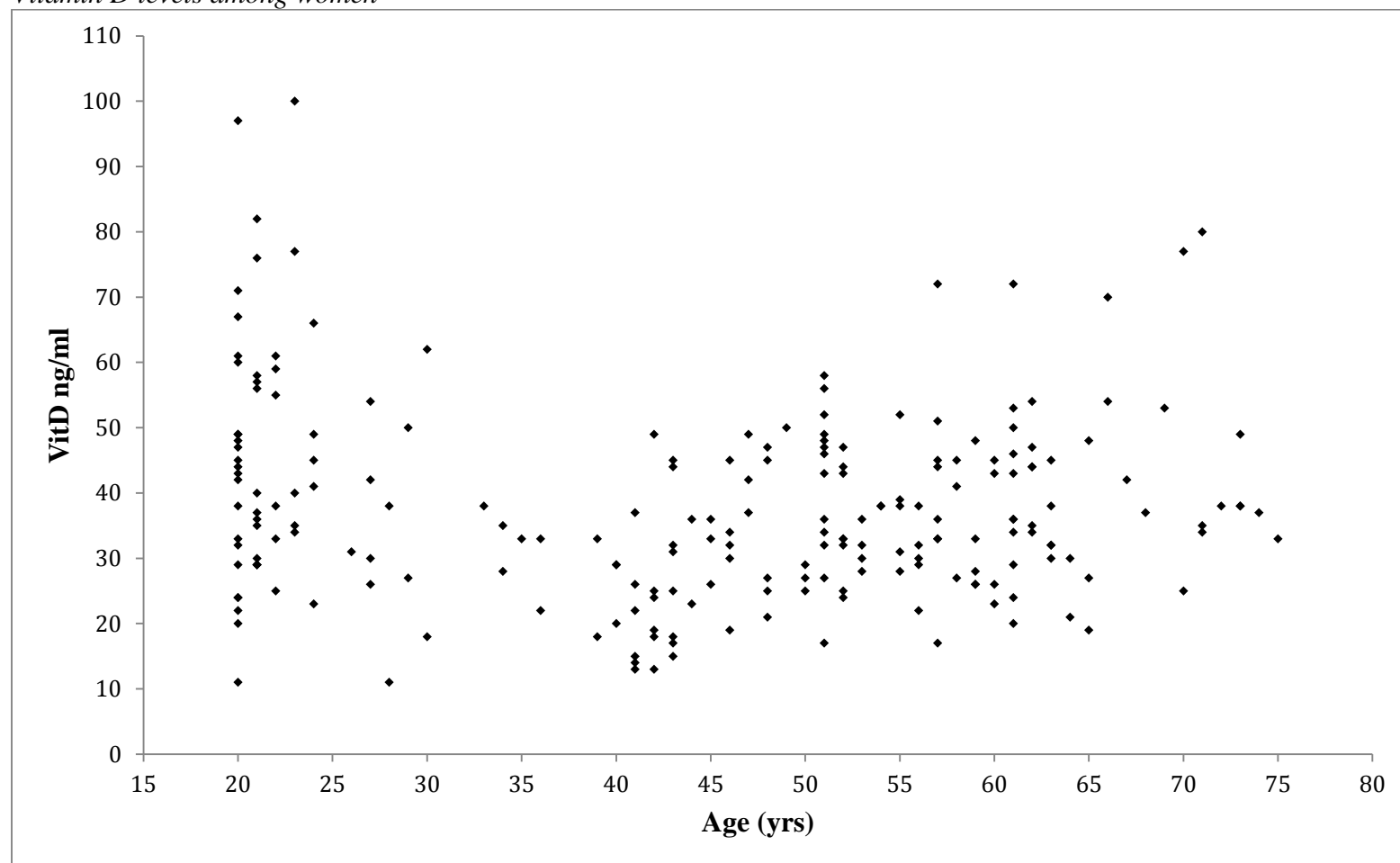
Chart 2.
Vitamin D levels among men



VitD, vitamin D; ng/ml, nanograms per millileter; yrs, years

Chart 3.

Vitamin D levels among women



VitD, vitamin D; ng/ml, nanograms per millileter; yrs, years

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